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NUTRITION REVIEWS

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NUTRITION REVIEWS

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COMPARATIVE NUTRITION

Comparative nutrition is concerned with the similarities and dissimilarities among different species of animals with reference to nutritional characteristics. The qualitative similarities among the higher animals are well established: the different species are nourished by essentially the same organic and inorganic nutrients; they digest and metabolize these nutrients in essentially the same manner; their ability to transform one nutrient into another is subject to essentially the same limitations so that the indispensable nutrients of a complete diet are much the same for all. Furthermore, the penalties of undernutrition with reference to any particular nutrient are remarkably similar among different species of animals. Qualitative dissimilarities exist, but are relatively few in number, and, in so far as they relate to the essential components of a complete diet, are frequently referable to the different synthetic capacities of the gastrointestinal flora and fauna rather than to differences in the hosts themselves.

The quantitative similarities and dissimilarities in the nutritional characteristics of the different species of animals are of great importance to the science of nutrition in the establishment of its general principles and laws and in the elucidation of causal relations. The surface area law associated with the name of Rubner was one of the first attempts to define a quantitative similarity among different species of animals with reference to a nutritional characteristic, the basal expenditure of energy. The relative constancy in basal metabolism, when expressed per unit of body surface, for adult warm blooded animals is a striking phenomenon, even though more accurate empirical methods of predicting the basal metabolism of animals have later been proposed by Kleiber (*Hilgardia* **6,** *315 (1932)*) and Brody (*Mo. Agr. Exp. Sta. Res. Bull.* **166** *(1932)*). Kleiber (*Proc. Soc. Exp. Biol. Med.* **48,** *419 (1941)*) and Weymouth, Field, and Kleiber (*Ibid.* **49,** *367 (1942)*) have also presented some evidence for the thesis that excised tissues from different species of animals, while preserving their relative relations, exhibit metabolic rates paralleling those of the animals from which they are derived.

The Terroine-Smuts (*J. Nutrition* **9,** *403 (1935)*) relationship between basal heat and the minimum endogenous loss of nitrogen is another striking illustration of a general nutritional principle of interspecific significance. For many adult animals, including man, approximately 2 mg. of endogenous nitrogen are inevitably lost in the urine per calory of basal heat. This loss measures the minimal protein requirement for maintenance in terms of net protein, and its relationship to the basal metabolism proves that the protein requirement of the adult organism varies more nearly with body surface than with body weight. The effect of sex and age on this ratio is yet to be precisely determined. The maintenance requirements of water by adult animals also seem to be closely related to body surface among animals varying in size from rat to man (Richter, *Am. J. Physiol.* **122,** *668 (1938)*). In unpublished work by Longwell from this laboratory, it appears that the endogenous loss of calcium from adult animals (rat, rabbit, pig, and man) approximates 0.14 mg. per calory of basal heat.

The energy requirements of the body and the food energy consumed seem to determine the need for many essential nutrients throughout all species of the higher animals. The investigations of Levine, Remington, and von Kolnitz (*J. Nutrition* **6,** *347 (1933)*)

suggest as much for iodine, and Cowgill's classic experiments on "vitamin B" ("The Vitamin B Requirement of Man," *Yale Univ. Press, New Haven, Conn.* (*1934*)) constitute weighty evidence for what, in all probability, is thiamine. Later studies on pure preparations of this vitamin with various species indicate that all animals may require about 1 p.p.m. of thiamine in the nonfat dry matter consumed, or 0.25 mγ. of thiamine per 1000 nonfat calories. Adolph (*Physiol. Rev.* **13,** *336* (*1933*)) assesses the water requirements of animals at 1 ml. per calory of heat produced. This seems to be a good approximation, except for animals consuming excessive amounts of protein or minerals, or, like ruminants, poorly digested food, or for man under conditions of profuse sweating. From this evidence and from many indirect considerations (Mitchell, *Federation Proceedings* **3**, *193* (*1944*)) it appears "that the body's primary need for nutriment is for food energy, and that its need for most of the specific essential nutrients is in proportion to the amount of food energy consumed." The functions of many of the vitamins and of the trace minerals seem to be involved in the transformations, syntheses, and oxidations of the organic nutrients upon which the body relies for its supply of energy. A prominent exception to this statement is the need for vitamin A, which Guilbert, Howell, and Hart (*J. Nutrition* **19,** *91* (*1940*)) found to be related to body weight among a wide variety of animals, an important finding that has been shown to apply to still other species of animals. This is the only nutrient the requirement for which has been definitely shown to vary with body weight rather than with body surface or some power function of body weight less than one. Wolbach's (*Science* **86,** *569* (*1937*)) studies of the functions of vitamin A have supported the suggestion "that vitamin A may be solely concerned in maintaining an apparatus within cells and not in the chemical processes for which the apparatus is necessary." This suggestion is in harmony with the generalization of Guilbert and associates.

Many of the phenomena of growth are of general occurrence among all animals: the shape of the growth curve except for man (Brody, *Mo. Agr. Exp. Sta. Res. Bull.* **104** (*1927*)), the relative growth of the different parts of the body (Huxley, "Problems of Relative Growth," *The Dial Press, New York* (*1932*)), the chemical development of the body (Moulton, *J. Biol. Chem.* **57,** *79* (*1923*)), and the relative accretions of its different chemical components (Needham, *Biol. Rev.* **9,** *79* (*1934*)). It appears highly probable that at equivalent ages in the Brody sense (*Growth* **1,** *60* (*1937*)) the growth accretions of all animals are of similar chemical composition on the protoplasmic (fat-free) basis.

However, the quantitative similarities among animals with reference to the nutrients required to maintain the *status quo* of the body on the one hand, and to promote growth on the other hand, do not imply quantitative similarities in the total nutrient requirements of growing animals of different species. The relative rate of growth, expressed by the ratio of growth rate, $\frac{dW}{dt}$, to body weight, W, determines the predominance of one or the other type of requirements. Thus, in the child during the juvenile period of growth, the relative growth rate is about 0.03 per cent per day, while in the rat it may be one hundred times as great, 3.0 per cent. The growth requirements are dominant in the latter case, but subordinate in the former. For this reason practice of testing with the growing rat the adequacy of human diets is inherently at fault. Furthermore, the relative intensity of the nutrient requirements of growth may be causally associated with the relative efficiency of nutrient utilization. The high efficiency with which the rat utilizes dietary protein and calcium, for example, as compared with man, may be an essential element in its survival through the ages on a food

supply not specifically adapted to the requirements of any one species.

The existence of striking similarities in the nutritional characteristics of different species of animals should not blind one to the dissimilarities, as Benedict ("Vital Energetics," *Carnegie Inst. Wash. Publ. no.* **503** (*1938*)) emphasizes in his study of comparative basal metabolism. Individuality among animals, functional variability under identical environmental conditions, and adaptability to different environments and to different food supplies (Mitchell, *J. Am. Dietet. Assn.* **20,** *511* (*1944*)) are prominent characteristics of living organisms. These are, perhaps, the exceptions that "prove the rule" and reveal important biologic principles.

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PARENTERAL NUTRITION IN MAN

It has become increasingly evident in recent years that the parenteral administration of food has a definite place in the practice of medicine and surgery. This type of feeding appears to be desirable in all conditions where oral alimentation is impossible or is contraindicated.

Of the six essential nutritional elements—water, electrolytes, carbohydrate, fat, protein, and vitamins—all have been given parenterally at least on occasion. Elman and Weiner (*J. Am. Med. Assn.* **112,** *796* (*1939*)) administered protein hydrolysates intravenously to patients, and Elman and associates are among the leading proponents of this type of alimentation. The conditions under which deficits of the essential nutritional elements may accumulate and under which parenteral feeding is indicated have been discussed by Elman (*Bull. N. Y. Acad. Med.* **20,** *220* (*1944*)). He states: "The first and most obvious indication for parenteral feeding includes patients unable to take any food or fluid by mouth because of vomiting, obstruction, etc. Second are patients in whom the utilization of food by mouth is incomplete or unsatisfactory, as for example, patients with severe diarrhea or with intestinal fistula. Third are patients in whom oral ingestion of fluids or food is actually deleterious, such as those with peritonitis or other inflammatory disease, or those operated on, in whom complete rest of the gastrointestinal tract is important. Fourth is an ill-defined group in which for one reason or another, usually severe anorexia, the oral intake of fluid or food, while possible, is inadequate. While the intake of food may be increased in these patients by gavage feedings, such a procedure is often followed by vomiting or diarrhea. In this group of patients it may be extremely important to supplement the oral intake by parenteral injections. Fifth, parenteral administration of food may prove important where time is limited for correction of nutritional depletion. For example, in preparing extremely malnourished patients for operation, the parenteral route may enable a nutritional deficit to be corrected even partially in a few days rather than in a few weeks, and this may greatly shorten the length of convalescence."

Elman suggests that despite the considerable evidence that fats supply more than calories to the growing organism and the fact that they are a vehicle for the fat soluble vitamins, it is probably unnecessary to supply them in short periods of parenteral nutrition. Should it prove desirable at some time in the future, however, the intravenous injection of homogenized fat appears possible (Holt, Tidwell, and Scott, *J. Pediatrics* **6,** *151* (*1935*)). There is also on record a case of a seventeen day period of parenteral nutrition of an adult male by

means of intravenous injections of glucose, casein digest, and emulsified fat and intramuscular injections of liver extract (Clark and Brunschwig, *Proc. Soc. Exp. Biol. Med.* **49,** *329 (1942)*).

As for deficits of vitamins, it is well known that severe acute deficiencies frequently may be corrected in a few days by parenteral administration of the appropriate materials.

Until recently it was assumed that deficits of protein were comparatively unimportant and could be met for long periods of time from nonessential stores such as "deposit" protein and, to some extent, muscle proteins. It now appears, however, that there is not a selective exhaustion of "nonessential" proteins. On the contrary, such proteins as those of plasma and liver are depleted whenever there is an insufficient protein intake (Weech, Goettsch, and Reeves, *J. Exp. Med.* **61,** *299 (1935);* Elman and Heifetz, *Ibid.* **73,** *417 (1941)*). It is unsafe to assume, therefore, that a protein deficit is unimportant. Long periods of inadequate protein intake are not uncommon and in such patients the deficits frequently reach high figures. These protein deficits, unlike those of water, electrolytes, and vitamins are difficult to correct and require long periods of therapy.

For the correction of protein deficits by the intravenous route, intact proteins, protein hydrolysates, or mixtures of pure amino acids may be employed. The choice of material will be governed largely by its effectiveness, availability, nutritional quality, cost, and by the presence or absence of untoward reactions. The use of intact proteins has been limited almost exclusively to those found in homologous plasma and these have been used only with certain types of patients. Many workers have employed protein hydrolysates, usually prepared from casein. Madden, Whipple, and co-workers have proposed the use of a mixture of pure amino acids.

The one great advantage of plasma is that it supplies preformed protein immediately to the circulating blood. For this reason it is used in cases of shock or where there is reason to fear the development of shock. For general use in intravenous alimentation, plasma has the disadvantages of expense (1 liter of plasma requires 4 donors and contains but 60 g. of protein) and of contamination with sodium citrate. Plasma may at times contain other undesirable contaminants also as evidenced by the recent cases of infective jaundice among army personnel. In addition, the proteins in plasma must be broken into smaller units before they can be utilized by other tissues of the body and there is doubt that they contain the essential amino acids in the most advantageous proportions. Holman, Mahoney, and Whipple (*J. Exp. Med.* **59,** *269 (1934)*) reported experiments with dogs in which the protein in plasma transfusions apparently supplied all body needs, but Daft, Robscheit-Robbins, and Whipple (*J. Biol. Chem.* **123,** *87 (1938)*) later demonstrated that when larger amounts were injected a large loss of nitrogen occurred in the urine with resultant negative nitrogen balance.

A protein hydrolysate is fairly inexpensive and represents a form not greatly different from that in which protein nourishment normally enters the blood. By proper choice of the protein to be hydrolyzed and the method of hydrolysis, a solution containing considerable amounts of all of the essential amino acids may be obtained. Some untoward reactions have been noted but possibly no more than with blood transfusions or intravenous injections of other types. Too rapid a rate of injection or the use of too concentrated solutions may cause flushing, headache, backache, abdominal cramp, nausea, and vomiting. A pyrogenic reaction may result from contaminated solutions or from other more obscure causes. Phlebitis and thrombosis may occur but, it is claimed, in no higher incidence than with glucose solutions alone. In all cases of cir-

culatory insufficiency, of course, intravenous alimentation, in common with other forms of intravenous therapy, must be used with caution.

The clinical results obtained with protein digests have been encouraging.

Madden and co-workers (*J. Exp. Med.* **77,** *277 (1943)*) proposed the clinical trial of a mixture of the ten essential amino acids plus glycine. The ten amino acids which are regarded as essential are threonine, valine, leucine, isoleucine, lysine, tryptophane, phenylalanine, methionine, histidine, and arginine. Madden *et al.* reported that this mixture is highly effective in plasma protein regeneration in dogs and is better tolerated than any protein digests which they had tested. Madden, Woods, Shull, and Whipple (*Ibid.* **79,** *607 (1944)*) confirmed and extended these observations. They suggested that the undesirable vomiting and pyrogenic effects caused by protein hydrolysates and certain amino acid mixtures may be due to nonessential amino acids and indicated that glutamic acid is such an offender. They found no evidence of toxicity of the unnatural forms of the synthetic amino acids. Bassett, Woods, Shull, and Madden (*New Engl. J. Med.* **230,** *106 (1944)*) reported a favorable clinical trial, in one patient, of the amino acid mixture of Madden *et al.* The greatest present deterrent to the clinical use of mixtures of pure amino acids appears to be their cost and limited availability. It is possible that large scale production may eliminate this disadvantage.

After a thorough and critical survey of available data concerning intravenous alimentation with protein digests and mixtures of amino acids, Gaunt (*Nutrition Abstr. Rev.* **13,** *501 (1944)*) concluded that it is a practice based on sound physiologic principles. He stated: "By eliminating the processes of digestion and absorption, intravenous injection of protein hydrolysates offer a physiological method of dealing with the alimentation of human subjects in a wide variety of pathological conditions. The clinical evidence so far obtained has shown that the intravenous administration of such materials can be helpful when oral feeding is impossible, difficult, or ineffective."

THE SIGNIFICANCE OF PERLÈCHE

The term perlèche until recently was employed solely in the vocabulary of dermatologists. Since the reports of Sebrell and Butler (*Pub. Health Reports* **53,** *2282 (1938);* **54,** *2121 (1939)*) this term has become popular and many have gained the impression that perlèche is synonymous with ariboflavinosis. Finnerud (*J. Am. Med. Assn.* **126,** *737 (1944)*) in a paper delivered at the last session of the American Medical Association discusses the nosology of perlèche.

The term perlèche is derived from the French "pourlécher" meaning to lick, since persons having the disorder frequently moisten the affected areas with the tongue, a symptom which is often of some etiologic significance. The subjective symptoms of perlèche are mild, usually consisting merely of a feeling of dryness and at times a slight burning sensation at the corners of the lips. Deep, infected fissures may be painful, and though rarely encountered, occur chiefly in perlèche of adults. Objectively, one first sees the epithelium of one or both angles of the mouth to be mother-of-pearl colored, somewhat macerated, either adherent or easily detached, and wrinkled; later the wrinkles become deeper, often forming usually one, sometimes multiple, transverse fissures with red bases, these fissures having little if any tendency to bleed. The involvement usually stops rather abruptly just within the mucocutaneous junction of the commissure but extends as a localized

erythematous scaling dermatitis, usually with fissuring from a few millimeters to as much as 2 or more centimeters outward and downward from the mouth angle onto the skin.

A number of observers have noticed that perlèche may occur under circumstances which are probably not related to riboflavin deficiency. For example, Ellenberg and Pollack (*J. Am. Med. Assn.* **119**, *790 (1942)*) studied a group of 34 patients with what they termed perlèche, all except one of whom were aged from 40 to 72 years and none of whom had other symptoms of avitaminosis. Thirty-two of the 34 patients had upper and lower artificial, ill fitting dentures, which resulted in a mechanical defect. The other 2 patients with perlèche, who did not have false teeth, had mechanical defects to explain the lesions: one a woman aged 26, who presented malocclusion and the other an edentulous man aged 72 whose upper lip sagged so as to overlie the lower. These authors were able to demonstrate that the lesions in their cases were not due to specific allergy to the components of the dentures or to the presence of impurities in undercured vulcanite plates, but definitely resulted from improper oral mechanics, in particular from an improper too short vertical dimension. As a result of the vertical dimension being too small, overclosure develops and the upper lip overlaps the lower, thus producing creases at the corners of the mouth and sagging. In this way a pocket formation occurs at the lateral angles of the lips, a receptacle for saliva which becomes a medium for fungus growth. Ellenberg and Pollack regard these fungi as causative in the production of the cutaneous lesions. It has been noticed that perlèche lesions usually do not appear until after dentures have been worn for as long as several years, during which time bone resorption is known to take place. A number of other investigators have made similar observations.

Finnerud (*Arch. Dermat. Syph.* **20**, *454 (1929)*) reported a study of over 100 children with perlèche, all from the same institution, in 77 per cent of whom certain fungi were isolated from the lesions. It was suggested at that time that these organisms were the cause of the lesions characterized as perlèche. Similar findings were recorded later by other writers. The nutritional status of these children was not studied.

Lesions having the appearance of those described in riboflavin deficiency have also been observed in instances of contact dermatitis, neurodermatitis, seborrheic dermatitis, atopic dermatitis, severe chapping, herpes, congenital syphilis, and in other conditions which, because of their localization in a position to include the oral commissures, and due to movement in this area during mastication and talking, cause a perlèche-like fissuring.

It might be argued by some that whenever lesions appear in the commissures of the lips, their development may depend on an underlying basis of riboflavin deficiency with the mechanical or traumatic factor acting as a secondary or inciting agent. That this explanation is not very likely is suggested by the fact that many observers have treated such lesions with riboflavin without benefit. It needs to be emphasized that perlèche is merely a symptom and like other symptoms of disease it may be caused by many factors, only one of which is ariboflavinosis. The indiscriminate treatment of all instances of perlèche by riboflavin is not consistent with the careful scientific work from which our knowledge about the vitamins has been derived.

EFFECTS OF GLUTAMIC ACID ON THE CENTRAL NERVOUS SYSTEM

Glutamic acid, a nonessential amino acid, has received recent attention as a therapeutic agent in the petit mal type of epilepsy and as a substance capable of augmenting learning ability in rats.

Petit mal is characterized by brief lapses of consciousness lasting only a second or two, but they may be repeated as many as fifty to a hundred times a day. Until recently, only two types of treatment have been even partially effective. These were sedation with long-acting drugs such as bromides, or the production of mild ketosis with a high fat diet. The effect of the ketogenic diet was attributed to the concomitant acidosis. In an attempt to achieve the supposed beneficial effect of acidosis without the inconvenience and possible hazards of the ketogenic diet, Price, Waelsch, and Putnam (*J. Am. Med. Assn.* **122,** *1153 (1943)*) added glutamic acid hydrochloride to an otherwise normal diet, assuming that an acidosis would result. They were gratified to find that patients with petit mal were indeed benefited by the glutamic acid, but the improvement could not be explained by the acidosis, which proved to be extremely mild (Waelsch and Price, *Arch. Neurol. Psychiat.* **51,** *393 (1944)*).

The glutamic acid therapy reduced the number of daily seizures to 10 or 15 per cent of their initial frequency, but was not completely effective. An increased mental and physical alertness was also noted in these patients. Following withdrawal of the amino acid from the diet, the frequency of seizures increased and could be again decreased by once more feeding glutamic acid. In contrast to the effect on petit mal, the grand mal type of epilepsy was, if anything, made worse with glutamic acid.

Two research groups have now tested the influence of glutamic acid on the learning ability of rats. Zimmerman and Ross (*Arch. Neurol. Psychiat.* **51,** *446 (1944)*) found that the incorporation of 200 mg. l-glutamic acid per day in the diet reduced the time required to learn a maze to a third or a fourth of that required by the controls. The results of these experiments, which involved over 100 animals, were statistically significant.

The amino acid glycine, in contrast, was without influence. In spite of its effect on learning, the added glutamic acid did not accelerate growth in these rats. Albert and Warden (*Science* **100,** *476 (1944)*) found that in addition to learning more rapidly, rats given glutamic acid could master a more complicated problem than control rats. In the test they used, the experimental animal, in order to secure food, must step on a series of plates on the cage floor in a given sequence. The rat is taught as long a sequence of plates as possible by increasing the number of plates one at a time. Of 8 control rats on a "well balanced" diet, only 4 could master a 2-plate sequence, and only 2 could solve a 3-plate sequence, with none of the 8 progressing beyond this point. In contrast, all 9 animals receiving a supplement of 150 to 250 mg. l-glutamic acid per day solved the 2-plate sequence; 8 solved the 3-plate, and 1 mastered both the 4-plate and 5-plate sequence, a rather remarkable feat. Although only relatively few animals were involved, these findings suggest that glutamic acid enhances, not only the speed of learning, but the final level of performance as well.

Waelsch and Price express surprise that glutamic acid, an amino acid which the body can readily synthesize, should have any specific influence at all, especially since as little as 12 g. of racemic glutamic acid hydrochloride (equivalent to 5 g. of the natural l-glutamic acid) was effective in man—this in spite of the fact that nearly twice this amount was already present in the protein of the diet. These authors point out, however, that an effect of glutamic acid on the brain is not without

precedent. L-glutamic acid is the only amino acid capable of maintaining the oxygen consumption of brain slices (Weil-Malherbe, *Biochem. J.* **30,** *665 (1936)*). Furthermore, Nachmansohn, John, and Waelsch (*J. Biol. Chem.* **150,** *485 (1943)*) found that choline acetylase which had been inactivated by dialysis could be reactivated by either glutamic acid or citric acid, whereas other amino acids were either inactive or only weakly so. Choline acetylase is an enzyme which synthesizes acetyl choline, the substance possibly responsible for both synaptic and nerve transmission. These *in vitro* effects of glutamic acid on brain slices and the brain enzyme choline acetylase suggest the possibility that glutamic acid may exert its effect in the intact animal by improving the metabolism of the brain.

It is thus not unlikely that glutamic acid may find uses of much more importance than the treatment of petit mal. At the present time only limited data on the use of glutamic acid have been reported for man and experimental animals. This will no doubt be promptly remedied. In addition to the problem of reproducibility of results, a number of other questions arise. Are any of the other amino acids or substances such as citric acid effective in petit mal or of influence on rat learning ability? Would an increased intake of proteins have the same effect as glutamic acid? Proteins, e.g. lactalbumin, contain as much as 25 per cent glutamic acid. Would withdrawal of the glutamic acid initially present in the diet have the reverse effect? Is the reported increased alertness in man following glutamic acid administration demonstrable objectively? The answers to these questions would help to establish the value of glutamic acid in human nutrition. To obtain these answers will require the unfortunately rare collaboration of nutritionists and psychologists.

THE DIETARY FACTOR IN THE ETIOLOGY OF PERNICIOUS ANEMIA

Long before modern methods of classification of the anemias were so highly developed, observant clinicians noted remissions and relapses in pernicious anemia. Improvement might occur in vegetarians when they ingested a diet high in red meats and liver. Following Whipple's experiments on blood regeneration in dogs, the investigations of Minot demonstrated the successful therapeutic use of liver and extracts of liver in pernicious anemia. This was followed rapidly by Castle's experiments on the mechanisms involved in the maturation and release of red cells. The concept of hematopoiesis which arose from his observations may be summarized as follows: A maturing factor required for erythrocytes (E.M.F.) is normally formed by the interaction in the alimentary canal of (1) an extrinsic factor from ingested food and (2) an intrinsic factor proliferated by the normal stomach. The E.M.F. is stored chiefly in the liver, but occurs in brain and kidney. It is relatively stable to heat and soluble in water and 70 per cent alcohol. The extrinsic factor occurs in substances rich in B-complex vitamins; it is heat stable and water soluble. The intrinsic factor occurs in normal gastric juice, is heat labile and is destroyed by peptic digestion. It is known not to be hydrochloric acid or any of the recognized digestive enzymes.

With this information in mind, many studies on the nature of the extrinsic factor have been made. After the discovery, isolation, and synthesis of all components of the vitamin B-complex so far characterized, it has been shown that none is the long sought extrinsic factor, nor does it appear to be any combination of them (Vilter, *Med. Clin. North America* **27,** *467 (1943)*). Some highly purified and potent extracts of liver

are said to contain only polypeptides, but their nature has not been elucidated further. The main stumbling block in the way of more rapid progress has been the lack of a satisfactory experimental animal upon which to test the various fractions which can be prepared. The only satisfactory method of testing a substance and of standardizing its strength is by use of the human subject with pernicious anemia in relapse. Since such patients are uncommon, progress has been slow.

Another approach to the problem has concerned itself with the natural history of the disease, its racial, genetic, geographic, and climatic determinants. These have been reviewed by Askey (*Ann. Int. Med.* **21,** *402 (1944)*), who presents the question, "Can diet deficiencies produce either typical Addisonian pernicious anemia or the early essential objective changes in proper sequence?" This is answered in the negative by a series of further questions and answers. First, there is no correlation between the distribution of the disease and poor nutrition in the geographic sense. Whether this is true among the individuals suffering from pernicious anemia has not been demonstrated but there is no evidence that this is true. The difficulty of fixing a precise time for the onset of the disease makes difficult the reconstruction of the relation of dietary abnormalities to pernicious anemia for loss of appetite and dietary peculiarities may result from anorexia and taste perversions resulting from the disease rather than acting as its cause. To the question, "Can dietary deficiencies produce Addisonian pernicious anemia?" the answer is given in the negative. It must be remembered that the clinical picture can be exactly mimicked by a dietary deficiency of the extrinsic factor as was suggested by Wills and Evans (*Lancet* **II,** *416 (1938)*) and clearly demonstrated by Moore, Vilter, and Spies (cited by Vilter, *loc. cit.*). Nonetheless, it has not been demonstrated that protracted deficiency of extrinsic factor ever leads to a deficiency of the intrinsic factor furnished by the alimentary canal, and thus diet cannot be said to cause true pernicious anemia.

Nevertheless, the relationship of diet to pernicious anemia will always be of interest since a factor must be present in food to prevent a macrocytic, hyperchromic anemia which closely resembles pernicious anemia. The final mechanism for producing extrinsic factor deficiency anemia and intrinsic factor deficiency anemia (Addisonian pernicious anemia) appears to be the same, namely, an insufficiency of active E.M.F. The defects which result in a reduction of E.M.F. may be inherent (intrinsic factor failure) or may come from a failure to eat adequately of proper foods.

PLASMA PROTEIN METABOLISM BY ISOTOPE TRACER TECHNIC

The studies by Whipple, Madden, and associates of the behavior of the blood plasma proteins in health and disease are well known. They have shown that the adult dog can be kept in positive nitrogen balance, weight balance, and in health for weeks while receiving carbohydrate, fat, minerals and accessories by mouth, and plasma protein in suitable amounts by vein. These workers believe that there is a flow of plasma protein out of the circulation into the tissues and vice versa, and to this the expressive term, "dynamic equilibrium," has been applied.

A further communication from their laboratory by Fink and co-workers (*J. Exp. Med.* **80,** *455 (1944)*) deals with a study in which heavy nitrogen was used to label protein. They chose for such an experiment an amino acid in which the uptake of nitrogen from the body pool of available nitrogen is at a minimum. Such an amino

acid is lysine, according to Weissman and Schoenheimer (*J. Biol. Chem.* **140,** *779 (1941)*) and in accordance with unpublished studies of Fink and co-workers. The method employed was as follows: To a dog selected as an eventual donor of labeled plasma protein a complete allotment of the essential amino acids was fed or injected daily, the lysine being supplied by a product synthesized with an excess of heavy nitrogen in the epsilon amino position. During the period of labeled lysine feeding, blood was withdrawn periodically and the plasma separated and stored in a freezing cabinet for further experiments. The red blood cells removed from the dog were replaced by equivalent amounts of washed red cells. The plasma protein level was kept low by suitable bleeding to ensure maximum plasma protein production. The concentration of labeled nitrogen in the lysine of this withdrawn plasma protein eventually reached a high enough level so that the amount could be measured with considerable accuracy even after a several fold dilution with plasma protein of normal nitrogen content.

The authors show that nitrogen isolated from intact plasma proteins through deamination with nitrous acid is derived predominantly from the epsilon amino groups of lysine. The use of epsilon-tagged lysine and the above deamination procedure made unnecessary the routine isolation of lysine from the plasma protein samples, and therefore greatly decreased the size of the plasma samples needed and the time and effort required in preparing the samples for heavy nitrogen analysis.

Protocols are given for studies in 2 normal dogs, in one dog in which shock was produced by intestinal trauma, and in 2 dogs in which shock was produced by leg trauma. In the normal animals portions of the blood were withdrawn and were replaced by blood of which the plasma proteins contained labeled lysine. In the shocked dogs labeled plasma was injected intravenously. Blood samples were taken periodically thereafter and the heavy nitrogen content of lysine from the plasma proteins thereof was determined.

The most striking result in these experiments was the rapidity with which this labeled plasma protein disappeared from the circulation and was replaced by unlabeled protein from the animals' own reserves. In twenty-four hours some 50 per cent of the labeled protein administered had already been replaced in the circulation by protein containing normal lysine, that is, the normal content of heavy nitrogen. In both normal animals the labeled nitrogen concentration had fallen to 50 per cent in approximately twenty-six hours. The further fall to 25 per cent required five days. The curve of disappearance was not logarithmic. The authors interpret the curve in the 2 normal animals as representing two phases. The presence of a highly mobile fraction of tissue proteins, exchanging constantly with the plasma proteins, would tend to cause the curve of disappearance of labile plasma proteins to assume an initial steep phase. However, as the tissue protein built up a higher isotope concentration, the apparent rate of loss of plasma protein would be slowed down due to the return of labeled protein from this mobile tissue protein pool.

Since the curves observed in the normal dogs and those in the animals in shock were similar, it was difficult to prove directly a difference between normal and shocked dogs. Nevertheless, if the shocked animals had been able to mobilize quickly large quantities of protein from the circulation, one might have expected a considerably increased rate of dilution of the labeled plasma in circulation to occur in these dogs. In fact, if even a normal rate of exchange between plasma protein and tissue protein had continued during shock, the influx of this normal amount of unlabeled protein into the greatly decreased amount of circulating plasma might have been expected to increase the rate of dilution. The lack of evidence for any such marked change in the slope of

the heavy nitrogen lysine disappearance curve in the shocked dogs may be considered as evidence that the rate of mobilization of tissue protein into plasma protein is greatly reduced in shock. It has been observed that the rate of sodium exchange across the capillary membrane is greatly reduced in shock (Gellhorn, Merrell, and Rankin, *Am. J. Physiol.* **142,** *407 (1944)*).

These observations bring further support to the thesis of the "dynamic equilibrium" of tissue and plasma proteins. Furthermore, the data presented indicate that there is in the normal animal an active tissue mass of protein about equal in size to the mass of plasma protein, since the rapid dilution phase seemed to end near the 50 per cent level.

FOOD DETERIORATION AND PEROXIDATION

The term "food deterioration" includes the development in stored food products of "off-flavor" or "oxidized" flavor, discolorations, and bleachings, and in a modern sense also the destruction of vitamins and other nutrients. There is increasing evidence that many if not most food deterioration phenomena are due to peroxidation. Peroxidation, in contrast to direct oxidation, involves the intermediate formation of peroxide derivatives of unsaturated compounds. Such peroxides are themselves active oxidizing agents and may thus perform secondary reactions destroying susceptible vitamins and flavoring or coloring substances. The most common naturally occurring unsaturated materials in foods which are susceptible to peroxidation are the fats. Hence it is the foods relatively rich in fat, particularly dairy products, which present the greatest peroxidation and deterioration problems. In this class of foods peroxidation is most commonly tabulated as "rancidity," although the latter term may be used more broadly to include hydrolysis of the fats.

Natural fats typically exhibit a delayed period of peroxidation (induction period) preceding the maximum rate that is finally reached. Since highly purified fats show a lessened induction period, it is evident that natural fats contain agents which inhibit peroxidation (antioxidants). The study of peroxidation and antioxidants, both from the academic and immediately practical standpoint, has engaged the attention of many workers. From the large body of accumulated facts, only a few pertinent points and recent contributions can be mentioned here.

Antioxidants, in very low concentrations, lengthen the induction period by the mechanism, according to current theories, of interrupting a catalytic cycle of organic peroxides. As such, antioxidants usually fall into the class of mild reducing agents, commonly hydroxy-organic compounds. Among the natural antioxidants are ascorbic acid, phenolic substances, and vitamin E. The significance of the latter in protecting animal fats from peroxidation, both in storage and *in situ*, has been previously discussed (*Nutrition Reviews* **3,** *17 (1945)*). Probably more attention has been directed to the search for satisfactory synthetic antioxidants. The systematic attack on this problem in Great Britain is evidenced by publications directed by the Food Investigation Board of the Department of Scientific and Industrial Research. Banks (*J. Soc. Chem. Ind.* **63,** *8 (1944)*) has published a basic investigation outlining a less time-consuming method for routine studies of the efficiency of antioxidants. A homogeneous water emulsion of linoleic acid is employed and oxygen consumption of the mixture measured in the now common Warburg respirometer. Hematin is employed as an oxidation catalyst to shorten the induction period which would otherwise (at

30°C.) be excessively long. This type of test system seemed justified in that natural products such as milk, butter, margarine, meat, and fish all contain fat in some form of an emulsion with water. It is pointed out however that the effectiveness of a given antioxidant in one test system may not be strictly comparable to that in another system. Thus hydroquinone is an efficient antioxidant for dry fats and fat-water emulsions, but not for dry breakfast cereals. Banks has presented a useful evaluation of a long series of hydroxybenzene and hydroxynaphthalene compounds, but more significantly has initiated a search for antioxidants in natural products. In this first paper, extracts of flower petals were tested, and among these it was noted that a simple extract of the peony and rose petal yielded a material with powerful antioxidant properties. The activity was apparently not related to the anthocyanins present. There seems little doubt but that a systematic search for antioxidants in plant products will yield new and efficient substances, either directly or by revealing another type of chemical structure particularly suited for its antioxidant properties.

The provitamin carotene and vitamin A are themselves unsaturated fat-like substances which can be oxidized either directly or secondarily by peroxidized fat. Lovern (*J. Soc. Chem. Ind.* **63,** *13 (1944)*), again under the coordination of the British Food Investigation Board, has studied the direct oxidation of carotene in ethyl acetate solutions exposed to light, and of vitamin A in aqueous emulsions. In these systems antioxidants exerted their typical effects. Diphenylamine was an efficient antioxidant for carotene, while ethyl and butyl gallate, dihydroxynaphthalene, and alpha-tocopherol were particularly active protective agents for the vitamin A in margarine.

Other reports indicate the intensive search for suitable antioxidants to be used in a variety of food products. Williamson (*Food Research* **9,** *298 (1944)*) has for example studied the efficiency of some antioxidants in preventing the onset of "rancidity" of the fats present in skim milk preparations. Test substances were mixed with dried skim milk powder, and the mixture incubated for as long as three months with weekly analyses of the peroxide content of the fat extracted from the test mixture. Of the antioxidants examined, which included cereal grain preparations, mixed tocopherols, and hydroquinone derivatives, the substance thiourea was found to be particularly effective. The activity of thiourea was found to be dependent on the moisture present in the test mixture, and evidence was cited to indicate its ineffectiveness in dry fats. Thiourea has been advocated previously as an agent to prevent darkening of fruits during preparation for processing. The now recognized physiologic action of this compound on thyroid function should be considered before its ready acceptance as a protective agent to be incorporated into food products.

Fat peroxidation, although inhibited by selected compounds, may be accelerated by other agents likely to be found in food products. Perhaps the most common of these positive catalysts is copper. Copper has been proved to shorten the induction period of fat peroxidation in experimental test systems, and in industry has been sufficiently well recognized as an undesirable contaminant to have led to complete elimination of copper and its alloys in modern food processing equipment. Hussong and Hammer (*Food Research* **9,** *289 (1944)*) have illustrated the deleterious effect of copper contamination on the bleaching of butter and the specific flavor defects of "fishiness" and "tallowiness." Samples of butter showing these defects were correlated with their copper content. In one instance these defects were shown to be due to the wrapping paper employed. The incorporation of copper salts into the cream before churning clearly demonstrated the effect of copper

contamination in accelerating flavor defects in butter during storage.

These few reports are only illustrative of the increasing impetus given to the field of peroxidation and antioxidants. It is increasingly clear that future food research must direct considerable attention to peroxidation as a mechanism of food deterioration, and to preservation of the natural protective antioxidants during processing.

RIBOFLAVIN DEFICIENCY IN SWINE

As the components of the B-complex are better defined and become available in crystalline form, it becomes possible to produce experimental deficiencies of the various vitamins which can be more readily attributed to the lack of a single vitamin than has been possible heretofore. Ellis, Madsen, and Miller (*J. Animal Sci.* **2,** *365 (1943)*) described riboflavin deficiency in pigs fed a purified diet probably adequate in all respects other than riboflavin. They observed impaired growth, frequent vomiting, severe diarrhea, rough skin and hair coat, considerable secretion about the eyes, and a peculiar gait. Incoordination occurred in 2 out of 4 pigs and histologic changes were noted in the sciatic nerve in 3 of the animals and in the spinal cord in one.

In their studies of nutritional deficiency in swine, Wintrobe and co-workers (*J. Nutrition* **24,** *345 (1942)*) found that good growth and normal appearance and activity occurred in pigs given a highly purified diet supplemented with crystalline thiamine, riboflavin, nicotinic acid, pyridoxine, choline, and pantothenic acid.

Using a similar diet, except for the fact that vitamin-free casein was used instead of crude casein, and furnishing crystalline vitamin supplements with the exception of riboflavin, Wintrobe, Buschke, Follis, and Humphreys (*Bull. Johns Hopkins Hosp.* **75,** *102 (1944)*) studied the effects in 3 pigs. Signs of nutritional deficiency became apparent within two to three weeks. The animals failed to gain in weight and began to lose their normal well-rounded appearance. Occasionally diarrhea appeared but lasted only a day or two. It is noteworthy that appetite was well maintained. In all 3 pigs the gait became abnormal and was characterized as appearing stiff, mincing, and hesitant. The gait was unlike the "goose-stepping" gait which these workers have described in pantothenic acid and pyridoxine deficient pigs. On the skin a mottled, erythematous eruption appeared. This was most noteworthy on the snout, behind the ears, along the midline of the back and in the adjoining regions of the skin, in the area of the groin, and over the abdomen. The affected skin became scaly and reddish brown scabs formed. As the deficiency progressed, normocytic anemia of moderate degree developed.

Striking changes were observed in the eyes in that the eyelids appeared swollen and lens opacities developed. The lens changes were first observed *in vivo* with the ophthalmoscope but a few days later could be seen even with the naked eye in one of the animals. This eye was enucleated and a photograph as well as a slit lamp composite drawing of the eye is presented. A dense white wavelike opacity was present near the equator of the lens. Comparable methods of eye examination failed to reveal cataracts in the eyes of control animals, including inanition controls, or in animals with other types of B-vitamin deficiencies. Similar cataracts could not be found in the eyes of a large series of pigs obtained at a slaughterhouse. It is significant to note, in view of the findings in the rat and those which occur in man, that the cornea was clear and showed no vascularization.

The evidence that these changes were due to lack of riboflavin is only indirect since it

depends merely on the exclusion of riboflavin from the diet. The observations, however, are consistent with those made in the rat by several workers and, except for the cataracts, are similar to those described in the monkey by Waisman (*Proc. Soc. Exp. Biol. Med.* **55,** *69 (1944)*). The latter described a "freckled" type of dermatitis, incoordination of the limbs, and severe anemia. Waisman called attention to the absence of impairment of appetite, cheilosis, and vascularization of the cornea. He made no mention of lens cataracts.

The specific relation of riboflavin to cataract was demonstrated in the rat by Day, Darby, and Langston (*J. Nutrition* **13,** *389 (1937)*), but the thin cornea and shallow anterior chamber of the eyes of a small animal such as the rat might conceivably predispose the rat's lens to cataract under the influence of exogenous physical damage such as evaporation and temperature changes. It is, therefore, significant that a relationship of riboflavin to cataract has now been demonstrated in animals as large as swine.

The failure to gain weight at a normal rate, in spite of good consumption of a diet which they had found to support good growth when it was supplemented with riboflavin, led Wintrobe and associates to postulate poor food utilization in riboflavin deficiency. Mannering and Elvehjem (*J. Nutrition* **28,** *157 (1944)*) offer evidence that riboflavin plays an important role in the economy of food utilization in the rat.

OVEREATING, OBESITY, AND DIABETES

Many observations indicate that obesity is associated with diabetes, that overfeeding may lower tolerance for carbohydrate, and that a reduction in weight of an obese diabetic patient by dietary restriction may improve carbohydrate tolerance. It is by no means clear what endogenous factors complicate the picture of weight gain or weight loss under varying dietary regimens and what relationship the development of obesity *per se* has to altered carbohydrate tolerance. Advancement in knowledge of the integrated roles of various hormones in human physiology brings increasing appreciation of the complexity of factors affecting the clinical course of diabetes. While it is well known that many diabetic patients display evidence of endocrinopathy other than that of the islets of the pancreas, the relationship of associated hormonal abnormalities to the diabetic syndrome remain obscure. It is difficult to establish in fact whether apparent endocrinologic disturbances are causally related to various manifestations of diabetes or are merely concomitant.

Handelsman (*Am. J. Med. Sci.* **208,** *15 (1944)*) has studied the response to weight loss, with regard to fasting blood sugar levels and glucose tolerance, in 55 adult patients (over 40 years of age) with diabetes of varying severity and duration. The results indicated that loss of weight induced by dietary restriction was an important factor influencing improved carbohydrate tolerance in obese middle-aged people whose glycosuria and hyperglycemia were of recent onset. On the other hand, it was observed that weight loss occurring with unlimited eating was followed by a permanent diminution in glucose tolerance. Handelsman also observed that obese patients who neglected treatment for many years and remained obese lost their ability to regain their tolerance and became difficult to treat. They suggested that this difference might be ascribed to the existence of two types of obese diabetic patients, one gaining weight and the other losing weight as they overeat. Differences in severity of the diabetic state in these patients may reasonably explain

such occurrences, but associated endocrinopathies may also play a role.

Draper, Dupertuis, and Caughey (*Tr. Assn. Am. Phys.* **55,** *146 (1940)*) touched on this problem when they combined anthropologic and clinical studies of a large group of diabetic patients and reported a high degree of correlation of obese "pituitary" cases with mild diabetes and of the thin "pancreatic" type with severe diabetes. Newburgh and Conn (*J. Am. Med. Assn.* **112,** *7 (1939)*) reported studies of obese patients with hyperglycemia and glycosuria whose glucose tolerance tests became normal when their weights were reduced to an ideal level by dieting. Further study of such patients should lead to a better understanding of the exogenous factors of obesity caused by overeating and of endogenous (hormonal) factors influencing the development of obesity, in relation to the diabetic syndrome.

It is well known that diabetes is more severe the earlier the age of onset, and that the progression of the disease is slower when it is first manifested clinically at a later age (Colwell, *Arch. Int. Med.* **70,** *523 (1942)*). Likewise the development of obesity may have a different significance at an early age than it has in later life, quite apart from the concomitant presence or absence of diabetes. Handelsman arbitrarily selected 40 years of age as the dividing line between the "middle-aged obese" and young diabetics. It seems probable that obesity at later ages is ascribable to purely dietary factors more often than at earlier ages, whereas obesity developing during the years of adolescent growth is more apt to be influenced by hormonal deficiencies.

SOURCE OF FAT IN FATTY LIVERS

The source of the fat which occurs in fatty liver has been a subject of continued interest with its practical import in the dietary control of fatty livers. As early as 1938, Barrett, Best, and Ridout (*J. Physiol.* **93,** *367 (1938)*) employed deuterium labeled fat to determine the source of fat in fatty livers. By comparing the deuterium content of liver and carcass fat after feeding labeled linseed oil to mice fed diets deficient in choline, they concluded that (1) after a definite period of fat feeding, a large fraction of the liver fat was derived from the ingested fat, (2) after one or more days of fasting following the labeled fat ingestion period, the absolute amount of liver fat may still increase but its deuterium content decreases, hence under these conditions the liver fat was mobilized from the depots, and (3) the effect of injection of anterior pituitary extract was the transfer of depot fats to liver fat. When the mice were kept on a diet high in carbohydrate, but poor in fat and lipotropic factors, following an initial period of the labeled linseed oil feeding, the deuterium content of the liver fat fell in a few days below that of the depot fats. Under these conditions new liver fat must have been derived from carbohydrate.

These studies have been confirmed and extended by Stetten and Salcedo (*J. Biol. Chem.* **156,** *27 (1944)*) by the somewhat different technic of raising the deuterium level of the body water either by injection or feeding of isotopic water. In studying the fatty liver of choline deficiency, rats were kept on a diet of 10 per cent vitamin free casein, 84 per cent glucose monohydrate, 4 per cent salt mixture, and 2 per cent cellulose roughage, with added vitamins, and 0.2 ml. of linseed oil given every ten days. Control animals received in addition 0.5 per cent of choline chloride in the diet. After a preliminary period, each rat was given by injection 1 ml. of almost pure D_2O per 100 g. of body weight, and the drinking water was replaced by 2.5 per cent D_2O. Following four days on this regimen the rats were killed

and the livers and carcasses separately pooled for analysis. From the deuterium content of the fatty acids of the liver and of the depots was calculated the quantity of newly synthesized fatty acid formed during the four day period and taken up by the liver and the depot. About one half of the extra fatty acid in the liver of the choline deficient rats was found to be newly synthesized. In the deficient rats, the depots contained three to seven times as much new fatty acid as the livers, while in the rats given choline there was twelve to eighteen times as much. Since the earlier study of Stetten and Grail (*Ibid.* **148,** *509 (1943)*) showed that the degradation of fatty acids occurs at a normal rate in the choline deficient animal, these results suggest that the major site of fatty acid synthesis is the liver, and that in choline deficiency the transport of fatty acids from the liver to the depots is slowed down.

On the other hand, when cystine at a level of 0.5 per cent in the diet was fed together with choline at 0.0725 per cent, an accumulation of newly synthesized fatty acids in the liver was noted, but there was more new fatty acid in the depots than in the depots of control animals given no cystine. From this observation Stetten and Salcedo concluded that the fatty liver produced in this way is due to accelerated fatty acid synthesis and not to slowing of the transport of fatty acid from the liver to the depots. The authors liken this to the fatty liver "incident to thiamine administration." However such fatty liver is produced by choline deficiency, and the fact that if the animal is also thiamine deficient the fatty liver fails to appear can be accounted for by the decreased food intake and consequently the decreased metabolic turnover of the thiamine deficient rat. It has been demonstrated by Handler (*J. Biol. Chem.* **149,** *291 (1943)*) that any deficiency concurrent with choline deficiency will prevent the appearance of fatty livers if it prevents the animal from growing. In the same way, the fatty liver seen after feeding cystine is in fact a choline deficiency, and if the choline of the diet is raised above the marginal level used by Stetten and Salcedo the fatty livers occur.

Applying their technic to mice, Stetten and Salcedo were able to confirm the findings of Barrett, Best, and Ridout that the fatty acid in the livers of mice which had been injected with anterior pituitary extract had largely been transported from the depots. Stetten and Salcedo suggest that an advantage is to be gained by classifying fatty livers according to the source of the extra fat found in the liver, and they imply that evidence dealing with fatty liver in man is due to excessive mobilization of the depot fat and its movement to the liver. Until definite evidence can be obtained there seems to be no reason to believe that all cases of human fatty liver arise in the same way, although one may agree that the majority do indeed follow the mobilization of the depot fat. Experience with animals has shown that cirrhosis of the liver can be produced by choline deficiency, and although it remains to be proved whether human fatty livers and cirrhosis can be caused in the same way, this remains a possibility.

ATWATER SYSTEM OF CALORIC VALUES

Several systems have been employed for calculating the energy value of diets in different countries of the world. In the Atwater system, which is widely used in the United States and Canada, the energy value is calculated by multiplying the grams of protein, fat, and carbohydrate present in the diet by the factors 4, 9, and 4 respectively. The corresponding values for the Rubner system, which is used throughout Europe,

are 4.1, 9.3, and 4.1 but these figures for fat and carbohydrate represent values for heats of combustion and do not take digestion losses into account. The Accessory Food Factors Committee of the Medical Research Council (M.R.C. system) accepts the Atwater values for protein and fat, but for carbohydrates the Atwater factor 4 is applied only to the "available carbohydrate," namely, to substances such as starch, sugar, and dextrin which can be determined directly, after converting them into their starch equivalents.

The existence of these different systems makes it difficult for an accurate international comparison of dietary values, and the need for a single acceptable system for evaluating the caloric value of diets has become particularly apparent in connection with the activities of the United Nations Relief and Rehabilitation Administration (U.N.R.R.A.). In an editorial review (*J. Nutrition* **28,** *443 (1944)*), Maynard has examined the bases of the Atwater and the M.R.C. systems, and concluded that the former is suitable for international comparisons provided modifications are made appropriate to the dietary habits of the people of the countries in which the system is applied.

The Atwater system is based on 185 dietary studies on several groups of people in different areas of the United States (Atwater, *12th Annual Report, Storrs Agr. Exp. Sta. p. 69 (1899)*). Atwater classified the foods consumed into various groups such as meat, milk, cereals, sugar, and vegetables, and then calculated the percentages of protein, fat, and carbohydrate furnished by each group of foodstuffs. On the basis of 97 human digestion experiments, he next arrived at the average coefficients of digestibility of the proteins, fats, and carbohydrates in an average mixed diet. By multiplying the values for the heats of combustion by the coefficients of digestibility, he obtained metabolizable energy values for the constituents of the different foods, and these values were then averaged according to the proportions in which the various materials occurred in the American diet. The over-all average values arrived at in this way were 4, 9, and 4 calories per gram of protein, fat, and carbohydrate respectively. For many years these values have proved to be generally applicable for evaluating the energy value of American diets.

The Atwater system fails when applied to diets which differ either in quality or quantity from the average American mixed diet, and the system tends to overvalue the caloric content of either a vegetarian diet or a diet composed of less digestible foods. Attention has been given to this drawback by the Medical Research Council of the United Kingdom, which contends that the Atwater factors are not applicable to the wartime diets of England since the latter contain more vegetable protein and there is a large consumption of potatoes and of less digestible foods such as flour of 85 per cent extraction. Maynard has pointed out however that the M.R.C. system suffers from other and probably more serious drawbacks. In the M.R.C. system, Atwater's figures of 4 and 9 are used for proteins and fats, but the total carbohydrate factor 4 of Atwater is used only for "available carbohydrate" expressed as starch equivalents. However, Atwater obtained the figure "4" in digestion experiments wherein the carbohydrates which were actually available were utilized, and this figure is an average for mixed carbohydrates of both high and low energy value. Thus by applying the figure 4 only to available carbohydrate, the M.R.C. system virtually introduces a double deduction, since this factor has already been corrected for digestion losses and for carbohydrates of lower caloric value than for starch itself. In many foods, such as potatoes, nuts, vegetables, and pulses, significantly more digestible carbohydrates are present than those that make up the total of the available carbohydrates accord-

ing to the M.R.C. system. Thus, the M.R.C. factor for carbohydrate would appear to rest on questionable grounds and Maynard believes that more accurate conclusions can be obtained by suitably modifying the Atwater factor itself.

The American diet is characterized by flour of high extraction and by a distribution of animal and vegetable proteins in the ratio of approximately 60:40. But the Atwater factors are not applicable as such either to individual foods or to diets with different proportions of animal and vegetable proteins. Nevertheless suitable over-all factors for proteins, fats, and carbohydrates in a diet composed of 30 per cent animal protein and 70 per cent vegetable protein, for instance, can be obtained by making use of the original Atwater tables. For protein this factor then becomes 3.75 instead of 4. The original tables can also be used to compute average factor values appropriate for different groups of food materials such as animal products, vegetable fats, cereals of different extraction, nuts, and pulses. Thus average figures can be worked out for the diets characteristic of different parts of the world. This procedure is obviously not as simple as any of the existing single systems now in use, but it has the merit of being based on only one system, which, incidentally, has survived even modern physiologic evidence on the digestibility of foods.

COMPLEX CARBOHYDRATES OF FOODSTUFFS

The diet of the people of the Orient and especially those of China and India consists chiefly of foods of vegetable origin. Seeds like rice, millet, or soybeans, and different kinds of vegetables constitute the major portion of their diets. These foodstuffs are relatively high in that fraction recorded as "crude fiber" in the tables of proximate analysis as ordinarily conducted. Until recently the crude fiber fraction was thought to represent material which was very poorly digested and virtually unavailable as a food. It now appears, however, that the disappearance of crude fiber from the digestive tract is extremely variable and depends in part upon the proportion of cellulose and pentosans or hemicelluloses and also upon the presence of specific bacteria in the alimentary canal. Lignin does not appear to be digested at all, while the young nonlignified cell membranes of plants are much more easily broken down by bacteria. In fact, the digestibility of the fibrous fraction appears to be inversely proportional to the lignin content of the material and there is a growing realization that the fibrous foodstuffs may influence the biologic value of a diet appreciably. Thus Crampton and Maynard (*J. Nutrition* **15,** *383 (1938)*) have shown that the lignin, cellulose, and hemicellulose content of certain animal feeds gave a better idea of their biologic value than the mere estimation of crude fiber. Hummel, Shepherd, and Macy (*J. Am. Dietet. Assn.* **16,** *199 (1940)*) reported that the complex carbohydrates constituting the crude fiber may influence not only the nitrogen retention of children but also their acid-base and mineral balances.

These considerations made it desirable to determine the proportion of lignin, cellulose, and hemicellulose in the foodstuff consumed by Oriental people, whose diets tend to be high in crude fiber. Such a study has been made by Kung (*J. Nutrition* **28,** *407 (1944)*) who partitioned the crude fiber into its various constituents and tabulated the values for seventeen foodstuffs commonly used in Chinese diets. The values are recorded on both the wet and dry basis and for raw foodstuffs as well as for those cooked in the Chinese fashion.

Leafy vegetables like cabbage, mustard,

rape, and coriander (Coriandrum sativum) had a high moisture content of approximately 90 per cent, and they contained 1.4 to 3.2 per cent of lignin, 4.3 to 14.2 per cent cellulose, and 2.1 to 5.3 per cent of total hemicelluloses, calculated on the dry basis. Dried seeds like millet or yellow soybeans contained much less of these complex carbohydrates, while rice failed to contain any significant amounts, presumably because the samples analyzed were highly polished.

In many areas of China the vegetables listed in the table constitute the only available foods for the people and the values given indicate that if the energy and protein requirements are to be met by these vegetables alone, then the intake of complex carbohydrates will be eight to ten times larger than that on a diet which supplied the equivalent amount of protein with dried seeds like the soybean. In other words, the biologic value of the vegetable diet and particularly its adequacy as a source of energy may depend greatly upon the extent to which the complex carbohydrates are utilized.

SIGNIFICANCE OF FECAL EXCRETION OF THIAMINE

Recognition of the ability of intestinal bacterial types to synthesize vitamins has directed attention to the kind of diet which may influence bacterial synthesis, and to the even more pertinent question of the availability of the synthesized vitamins for human nutrition.

Ordinarily an increase in the amount of a vitamin ingested results in an increase in the amount excreted, but data have also accumulated to show that different non-vitamin components of the diet can also affect the amount of any particular vitamin excreted. The nature of the carbohydrate appears to be particularly important in this connection since the type of carbohydrate can influence the type of intestinal flora that will be maintained, and this indirectly affects the synthesis of essential vitamins. Williamson and Parsons (*J. Nutrition* **29,** *51 (1945)*) have carried out an extensive study of the amount of thiamine in the urine and feces as affected by diets containing high and low amounts of fiber. The basic diet of their subjects consisted of milk, butter, ice cream, eggs, cheese, and unenriched bread, and the supplements consisted of crushed drained pineapple (high fiber) or pineapple juice (low fiber). Both diets supplied 1.3 mg. of thiamine per day, and they were consumed by each subject for periods of five to seven days. From the data obtained with 6 subjects it appears that the average amount of thiamine excreted in the urine was approximately the same whether the high or low fiber diet was consumed (225 to 260 micrograms per day), but that fecal excretion of thiamine was markedly affected. When pineapple juice was fed, an average of 187 and 267 micrograms for two different series were excreted daily compared with 381 and 393 micrograms of thiamine when crushed pineapple was fed. This difference was accounted for by the larger amount of fecal material voided on the high fiber diet since the concentration of thiamine per gram of dry feces was the same for both groups. Since the thiamine content of both diets was the same, it was concluded that the fiber in the crushed pineapple had favored the microbiologic synthesis of thiamine. The possibility was also considered that the fiber in the crushed pineapple may have interfered with the absorption of the thiamine originally present in the diet, but the similarity in the amounts of thiamine in the urine of both groups of subjects would suggest that the extra thiamine in the feces was nondietary in origin.

In another experiment designed to test the effect of a diet consisting of milk, cheese, and eggs as compared with one consisting

primarily of meat (cured ham), Williamson and Parsons noted that the amount of thiamine excreted in the urine was simply proportional to the amount ingested, and the fecal excretions of thiamine on the two diets were the same.

Of greatest practical import is to know whether the thiamine synthesized by intestinal bacterial action is available to the host. Older observations question the nutritional significance of the synthesized thiamine since individuals suffering from beriberi may still excrete appreciable amounts of thiamine in the feces. A limited availability is also indicated in the work of Williamson and Parsons since increased fiber of the diet caused higher fecal thiamine excretion values without increasing urinary output of thiamine. On the other hand, the work of Najjar and Holt (*J. Am. Med. Assn.* **123,** *683 (1943)*; *Nutrition Reviews* **2,** *109 (1944)*), in which severe deprivation of thiamine was required to produce signs of thiamine deficiency, has been generally interpreted to indicate that bacterial synthesis of thiamine may play an important role in thiamine nutrition. This hypothesis was supported by the finding that retention enemas of 50 mg. of thiamine caused increased urinary thiamine excretions, proof that this thiamine was at least partially absorbed. The possible role of thiamine biosynthesis has of course caused much concern to those interested in thiamine nutrition and requirements.

However, the utilization of fecal thiamine and diphosphothiamine under normal circumstances has been questioned by Alexander and Landwehr (*Science* **101,** *229 (1945)*). These investigators analyzed for free and bound thiamine the stools of a normal individual on a 2400 calory diet. Free thiamine consisted of 10 to 20 per cent of the total, and more than half of this existed within the bodies of bacteria or other organisms in the feces. In order to determine whether the amounts of thiamine and diphosphothiamine present in the feces could be utilized, retention enemas containing twice the amount of free and phosphorylated thiamine as found in the average twenty-four hour stool, were administered. No increase in urinary thiamine excretion was noted, and all the administered material was recovered as such from the following twenty-four hour stool. It is pointed out that the two successive 50 mg. doses of thiamine used by Najjar and Holt are grossly unphysiologic doses as compared with the amount of thiamine normally present in the feces, and that actually most of the total thiamine in the feces is the bound form which cannot be absorbed as such.

It therefore appears that the utilization of fecal thiamine is very limited and can hardly cause serious errors in determining dietary thiamine requirements, although it cannot be denied that sufficient may be absorbed during severe dietary restrictions at least to prolong the onset of deficiency symptoms.

VITAMIN SUPPLEMENTS IN NORMAL PERSONS

There has been some disagreement as to whether the administration of vitamin supplements to persons consuming average diets can be justified. It has been argued that such vague symptoms as weakness, nervousness, fatigability, and insomnia can result from a vitamin deficiency and, therefore, when such symptoms appear vitamin therapy should be instituted (Haggard, The Nutrition Front, New York State Joint Legislative Committee on Nutrition, *Legislative Document no.* **64,** *(1943)*). It has been claimed that the average American diet often is not adequate to maintain optimal nutrition. The realization that there must be some stage between optimal nutrition and the well established deficiency states which can be treated effectively by

administering the substances which are lacking has stimulated a search for "subclinical" deficiency syndromes. It has been implied that even when no demonstrable deficiency exists one's sense of well-being and ability to perform work can be improved greatly by the addition of vitamins to the diet. There has been the hope too, in the minds of some, for the discovery of an easy way of treating the vague symptoms to which most of us are subject.

There has, however, been no conclusive evidence to substantiate the point of view that the administration of vitamin supplements to apparently normal persons is of value. In an effort to determine the effect, if any, of various vitamin supplements on apparently normal persons, Ruffin and Cayer (*J. Am. Med. Assn.* **126,** *823 (1944)*) carried out observations on 200 subjects. The subjects were volunteer medical students and technicians. They all continued their usual activities and ate essentially the same diet throughout the period of study which lasted thirty days. Each was given a worksheet to be kept daily and was instructed to record his impression as to the effect of the medication he was taking on the appetite, energy and "pep," general health, "gas" or indigestion, nausea, vomiting, the number of stools per day, abdominal pain, and weight. Extraneous factors which might possibly have affected the subjects were noted. A code was devised in an attempt to assure uniformity of recording. The experiment was done in midterm in order to avoid the factor of examinations. No subject knew what he was receiving or to which group he belonged, although they all knew that some would receive placebos and others various vitamins. They were instructed not to compare notes or to discuss their symptoms with fellow subjects.

The 200 subjects were divided into five groups. Group A received vitamin tablets plus liver extract tablets; group B received vitamin and yeast tablets; group C received vitamin tablets and placebos instead of liver or yeast tablets; group D received vitamin tablets only; group E received nothing but placebos. The content of the vitamin tablets was vitamin A, 2500 U.S.P. units; vitamin D, 200 U.S.P. units; thiamine chloride, 1 mg.; riboflavin, 1.5 mg.; ascorbic acid, 27.5 mg.; nicotinamide, 10 mg. Three of these were given per day. The liver extract tablets contained 0.5 g. of Wilson's liver extract 1:20. Six of these were given per day. The yeast extract tablets contained 0.5 g. of dried yeast extract. The placebos contained dextrose. The supplements used were of the same size and appearance so that identification was difficult if not impossible without chemical analysis. The tablets were coated with gelatin cane sugar, with iron oxide as a coloring matter.

It is stated that the greatest care was exercised in the evaluation of changes noted by the subjects. Only when these were definite and unmistakable were they recorded as such. The evaluation of the data submitted by each subject was made without knowledge of what supplements he had been taking. Statistical analyses of the data were made using the chi square test.

No essential difference was observed among the various groups in regard to appetite, energy and "pep," general health, or weight. However, in the group taking yeast there was a definite increase in the number of subjects who recorded the development of abdominal pain. It is well known that yeast and liver extract occasionally may cause distressing gastrointestinal symptoms and this was confirmed by the observations recorded in this study.

A simple study limited to the recording of subjective symptoms can hardly be thought of as an experimental investigation showing that vitamin supplements to average diets are without beneficial effect. No chemical or physiologic studies were done on the subjects, not even an attempt to evaluate the diets. It is well known that vitamin supplementation of adequate diets is without effect, particularly in "apparently nor-

mal" young adults. Therefore a paper such as the one covered in this review, although of popular interest, leaves much to be desired as a contribution to scientific nutrition.

DIET AND RIBOFLAVIN REQUIREMENT

It has long been suspected that the apparent requirement of the rat for vitamins of the B-complex may be altered by changes in other components of the diet (*Nutrition Reviews* **1,** *220* (*1943*)). Earlier investigations were handicapped however by a lack of the relatively refined technics available today. Rats can now be reared successfully on rations containing only purified constituents and synthetic vitamins, and furthermore there are rapid micromethods available for the determination of most of the vitamins. Often the newer methods are vastly more sensitive than the animal assays formerly used. Accordingly, the changes in the vitamin content of the food, the tissues, the excreta, and the contents of the digestive tract may now be readily and accurately measured.

Mannering, Orsini, and Elvehjem (*J. Nutrition* **28,** *141* (*1944*)) have made a detailed study of the effect of various basal diets upon the riboflavin requirement of the rat. In their original studies (Mannering, Lipton, and Elvehjem, *Proc. Soc. Exp. Biol. Med.* **46,** *100* (*1941*)) rats receiving borderline amounts of riboflavin grew more poorly when fed diets high in fat than when the fat was replaced by an isocaloric amount of dextrin. In other words, high levels of fat appeared to increase the requirement of the rat for riboflavin. Two possible interpretations of this effect of fat were offered: (1) that an increased cellular demand for the vitamin occurred during the metabolism of fat, or (2) that the higher levels of fat in the diet had altered the intestinal flora so that the rate of destruction of riboflavin in the gastrointestinal tract had been increased or that less than normal amounts of riboflavin were synthesized by the microorganisms.

The latter interpretation now appears to have been strengthened by the extended studies of Mannering *et al.* Weanling rats were fed purified rations in which the nature of the carbohydrate and the level of fat (lard) were varied. Adequate amounts of all the synthetic B-vitamins except riboflavin were supplied in the ration while riboflavin was fed by dropper at controlled, suboptimal levels. Under these conditions, rats which received a diet high in dextrin or corn starch showed a greater increase in weight than those which received rations containing sucrose, lactose, or a mixture of cellulose (Cell-U-flour) and sucrose. When part of the dextrin was displaced isocalorically by fat to the extent of 38.2 per cent of the ration, the rats grew no better than those receiving the sucrose diet. In other words, fat appeared to decrease growth. When lard was added to the diets in which the carbohydrate was sucrose, lactose, or a mixture of cellulose and sucrose, the fat did not decrease growth further. After six weeks of growth, the suboptimal supplements of riboflavin were discontinued entirely and following a two day interval, fecal collections were made for one week. For most of the diets the amounts of riboflavin excreted per week in the feces were found to be proportional to the rate of growth on the suboptimal levels of riboflavin. Approximately the same amount of fecal riboflavin was excreted weekly by the animals receiving high sucrose, fat-sucrose, fat-dextrin, or sucrose-cellulose, while on the diets containing dextrin or starch there was a twofold increase in fecal riboflavin. The greatest fecal production of riboflavin was observed on the diets containing lactose, although growth was relatively poor on these diets. Apparently, however, the factor limiting the rate of growth was something

other than the amount of riboflavin available, for relatively poor growth of rats on a diet containing 48 per cent of lactose and an abundance of all the known vitamins including riboflavin has been observed (*Nutrition Reviews* **2,** *267* (*1944*)). Ershoff and Deuel (*J. Nutrition* **28,** *225* (*1944*)) maintain that lactose may actually be toxic for certain strains of rats.

The inference from the experiments of Mannering *et al.* is that dextrin, corn starch, and lactose permit the development of intestinal organisms that synthesize more available riboflavin than that present when the carbohydrate is sucrose or when the percentage of fat in the diet is increased. Several investigators (*Nutrition Reviews* **1,** *220* (*1943*)) have noted that certain rations low in vitamins permit a greater rate of growth than can be accounted for by the vitamin content of the diet itself, and attempts have been made to correlate the unexpected growth with an increased vitamin content of the feces. It was believed that coprophagy might be necessary to obtain the benefit of the vitamins synthesized in the gut. But Mannering, Orsini, and Elvehjem now suggest that a portion of the vitamin (riboflavin in this case) synthesized in the large intestine by microorganisms may be available to the rat directly. Rigorous proof is difficult however, because deficient rats tend to resort to coprophagy even when kept in screen-bottomed cages. In any event, caution should be exercised in interpreting the results of vitamin balance studies, for the presence of riboflavin in the feces can no longer be considered as unabsorbed vitamin of dietary origin.

Among other effects of high fat on the symptoms of riboflavin deficiency was the observation that the amount of hair loss and the degree of dermatitis was greater on the diet high in fat than on the carbohydrate diets. A chronic deficiency in the presence of fat was characterized by a spastic paralysis of the hindquarters, a condition that did not occur with any marked regularity on the rations high in carbohydrate. The paralysis appears to be associated with the lesions in the nervous system described by Shaw and Phillips (*J. Nutrition* **22,** 345 (*1941*)), and might suggest a role for riboflavin in the metabolism of nerve tissue. Fat also appeared to hasten the death of riboflavin deficient rats. Of 7 rats fed the lard-sucrose diet without riboflavin, none survived longer than one hundred twenty-six days, and the average survival time was eighty-one days. On the other hand, 3 of 6 rats fed the high sucrose diet were still alive after one hundred thirty-three days.

Mannering and Elvehjem (*J. Nutrition* **28,** *157* (*1944*)) have also studied the effect of riboflavin on food utilization. The growth per calory of food consumed was calculated for various groups of animals which received graded, suboptimal levels of the vitamin. It was found that the young animals fed 12 micrograms of riboflavin per day gained at least eight times as much weight per calory consumed as those not receiving riboflavin; they gained about 50 per cent more per calory consumed as those fed 3 micrograms of riboflavin a day. In other words, the poor growth observed on diets low in riboflavin cannot be ascribed primarily to a decrease in food intake. As a matter of fact, adult animals deprived of riboflavin required about 1.6 times the amount of food eaten by restricted control animals to maintain the same body weight. A moderate loss of appetite as measured by food consumption was found to accompany a riboflavin deficiency, but curiously, the food intake often increased for a period of a few days just prior to death.

PARA-AMINOBENZOIC ACID ANTAGONISM TO SULFONAMIDES AND MARFANIL

Para-aminobenzoic acid (PAB) is one of several substances, first recognized as growth factors for microorganisms, which now are regarded as vitamins. The history of PAB, however, is in many ways unique. Its correct structure was predicted largely from theoretical considerations, several months before it was isolated from natural sources. At a time when this compound had not been shown to be a growth factor for any microorganism, it was postulated that it would prove to be an essential metabolite for bacteria in general. It was further predicted at that time that PAB would prove to act as a substrate in an anabolic enzyme reaction. The extent to which the bacteriologic and nutritional properties of this substance conform with those predicted for it has been the subject of numerous investigations within the past few years.

The discovery of PAB was a direct result of the use of sulfonamides as bacteriostatic agents. Woods (*Brit. J. Exp. Path.* **21,** *74* (*1940*)) and Woods and Fildes (*Chem. Ind.* (*London*) **18,** *133* (*1940*)) found that yeast extracts contained a substance which reversed the inhibitory action of sulfanilamide. From its chemical properties and its behavior in growth tests it seemed that this factor might be structurally related to the drug. It had been shown to have an acidic group, apparently carboxylic in nature. PAB was, therefore, tested and found to have strong antisulfanilamide activity. It was noted, also, that the chemical properties of the active component of the yeast extracts, so far as tested, corresponded to those of PAB. From these results, Woods postulated first, that the yeast antisulfonamide factor is PAB, second, that PAB is essential for the growth of bacteria but is normally synthesized in sufficient quantity by many strains of organisms and, third, that an anabolic enzyme reaction involved in the further utilization of PAB is subject to competitive inhibition by sulfanilamide or other sulfonamide, due to the structural similarity of the two compounds. This third postulate is often referred to as the Woods-Fildes theory of sulfonamide action.

The first two postulates have received experimental support; the third postulate is the critical portion of the Woods-Fildes theory. For this, there has never been any direct evidence, though there is much indirect evidence in its favor. One of the principal arguments against the theory rests on the observation that sulfonamides may inhibit systems of which PAB is not a component, yet the inhibition is still antagonized by PAB. For example, Sevag, Henry, and Richardson (*Am. J. Med. Sci.* **205,** *877* (*1943*)) observed that PAB counteracts the inhibitory action of sulfathiazole on the carboxylase activity of E. coli and S. aureus. Such experiments may question the specificity of the inhibitory action of sulfa drugs, but may nevertheless lend support to a general theory of competition between sulfa drugs and PAB. In support of the theory is the well established fact that the relation between PAB and sulfonamide is a competitive one, the observation that the presence of sulfonamide stimulates the production of PAB by microorganisms and may cause the concomitant appearance of a "sulfonamide-fast" strain, and the evidence for the presence of PAB in all cells. Furthermore, analogues of other growth substances have been shown to be active in inhibiting bacterial growth and this inhibition is antagonized by the growth substances in question.

O'Meara, McNally, and Nelson (*Nature* **154,** *796* (*1944*)) have supported the theory of sulfonamide-PAB antagonism and have provided evidence for a possible mechanism of the antagonism. They state that sulfanilamide acts on bacteria only when they are multiplying, that is, during the

logarithmic phase of growth. Due to the fall of potential in the medium during the growth phase, these investigators searched for a reducing agent, and found that at the time when a sulfonamide would be effective, a "dienol" compound, perhaps reductone, occurs in the medium. This substance, not yet positively identified, gave the O-dinitrobenzene test for dienol compounds. Reductone (prepared by alkali treatment of glucose) makes a complex with PAB and with sulfanilamide, sulfapyridine, or sulfathiazole. The complex with PAB was more soluble and easily hydrolyzed to the original components than the sulfonamide-reductone complex. The investigators postulate that the function of PAB is to condense with or temporarily stabilize reactive compounds of the reductone type, which are essential in metabolism and would without stabilization be lost to the bacterial cell. They support their theory with the findings that growth of streptococci occurred upon supplying the PAB-reductone to a medium otherwise deficient in energy sources, but not upon supplying the sulfapyridine or sulfathiazole-reductone compounds. Bacteria have also been shown to assimilate PAB during active growth phases, and return it to the medium at later stages of growth. The same occurs with sulfa drugs, but not as rapidly.

The properties of a sulfonamide of unusual structure, recently introduced into clinical use in England and in the United States, throws an interesting light on theories of the mode of action of the sulfonamides. In 1940, Miller, Sprague, Kissinger, and McBurney (*J. Am. Chem. Soc.* **62,** *2099 (1940)*) described the synthesis of p-sulfonamidobenzylamine. This compound was found to have no significant protective action against experimental streptococcal infections in mice and appears to have had no immediate clinical trial. Among other materials captured by the British from the Germans in the desert battles, however, was this particular compound and the important claim was advanced by the Germans that this drug is effective in the presence of pus. Called by the British "Marfanil" it was tested in the treatment of purulent wounds by Mitchell, Rees, and Robinson (*Lancet* **I,** *627 (1944)*). These authors quote Professor Fleming to the effect that this sulfonamide is not inhibited by PAB or pus (Fleming, *Special report on tests with Marfanil prepared for Medical Research Council (1943)*). The clinical trial by Mitchell and associates was outstandingly successful, especially considering the difficulties of treating infected wounds. A report is given on 22 cases. Four were burn cases and for first and second degree burns Marfanil does not show great promise. In 12 of the remaining 18 cases healing was almost certainly hastened and all of the wounds assumed a healthier appearance, with none showing epithelial destruction or exuberant granulations. In 6 cases, no growth could be obtained on cultures after four to twelve days' treatment despite the fact that these were purulent wounds not normally influenced by local chemotherapy. The authors concluded that of the many substances they had tested in the treatment of infected wounds, penicillin alone gave better results. In a further publication, Evans, Fuller, and Walker (*Lancet* **II,** *523 (1944)*) reported on the successful use of Marfanil and similar compounds in experimental gas-gangrene infections in guinea pigs. It appears that Domagk (*Klin. Wchnschr.* **21,** *448 (1942)*; *Deutsche med. Wchnschr.* **69,** *379 (1943)*) was the first to note the effectiveness of Marfanil against anaerobes both *in vitro* and *in vivo* and it is reported that this compound is generally used for prophylaxis in the German army. Evans and associates also noted in corroboration of the report by Fleming that this drug is not inhibited by PAB.

The failure of PAB to antagonize Marfanil is of great interest in a consideration of the mode of action of sulfonamides. Goldacre (*Nature* **154,** *796 (1944)*) has suggested that this failure may be due to the greater basic strength of the benzylamine derivative

as compared with the usual sulfonamides which are weak bases. If the forces between PAB or the sulfonamide and the compound (or enzyme ?) with which they combine are electric in nature, then this difference in basic strength of the new sulfa drug may explain its failure to compete with PAB. Marfanil is nevertheless antibacterial, and the failure of PAB to antagonize the action of this drug remains a challenge to the Woods-Fildes theory. It is conceivable of course that Marfanil competes with a different postulated growth factor, for example 4-aminomethyl-benzoic acid, the homologue of PAB which corresponds to Marfanil. This compound has not yet been tested however as a growth factor.

The elucidation of the mechanism of sulfonamide action will likely have a profound influence on our approach to chemotherapy and on certain aspects of nutrition. The recent contributions reviewed indicate the research interests in this problem, and offer new approaches to its solution.

NUTRITIONAL MACROCYTIC ANEMIA IN PATIENTS WITH PELLAGRA

In a certain proportion of patients with pellagra, anemia characterized by an increase in the average size of the red corpuscles is found. The frequency of this type of anemia varies and its pathogenesis has not been clear. For the first time it has been subjected to thorough study, as reported by Moore, Vilter, Minnich, and Spies (*J. Lab. Clin. Med.* **29,** *1226 (1944)*).

The 25 cases studied were selected from a group of 56 with macrocytic anemia seen in the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama. In these cases the anemia was severe and the diet had been grossly deficient in animal protein and vitamins of the B-complex for years. The subjects usually also had glossitis, pellagrous dermatitis, cheilosis, or peripheral neuritis. As is the case in sprue and in a form of macrocytic anemia seen in the tropics (Wills, *Indian J. Med. Res.* **21,** *669 (1934)*), the anemia in these cases of pellagra and the bone marrow were morphologically indistinguishable from those of pernicious anemia.

The present concept of the pathogenesis of pernicious anemia is based chiefly on the experiments of Castle (*Ann. Int. Med.* **7,** *2 (1933)*) which indicated that there is lacking in the stomachs of such patients not only free hydrochloric acid but also an "intrinsic factor" which normally is found in gastric juice. According to Castle's hypothesis, this intrinsic factor together with an "extrinsic factor" derived from food substances is absorbed through the intestinal tract and in some manner these two factors lead to the production of an erythrocyte maturing factor necessary for normal red cell formation. Known sources of extrinsic factor include beef muscle, milk, eggs, liver, yeast, rice polishings, and wheat germ.

Castle showed that in pernicious anemia the feeding of beef muscle is followed by no changes in the blood. Moore and associates found in 5 cases of pellagra with macrocytic anemia that the feeding of 200 g. of ground beef muscle daily for periods of seventeen to twenty-seven days was followed by a reticulocyte rise of 5 to 15 per cent; nevertheless, no significant rise in the erythrocyte count followed. The latter was attributed to the continued presence of diarrhea and of glossitis with resulting difficulty in taking food. When such complications were controlled in 6 other cases by first giving niacin and other crystalline vitamins as well as a more ample diet which nevertheless contained little or no "extrinsic factor," the feeding of an alcoholic extract of beef was followed by a reticulocytosis ranging from 7 to 31 per cent, and a significant increase in the erythrocyte count occurred. Thus it was shown that beef

muscle and its alcoholic extract contain some substance, presumably extrinsic factor, which was lacking in the diet of the cases of macrocytic anemia in pellagra.

The persistence of more or less diarrhea in spite of administration of niacin, as well as the development of a more pronounced reticulocyte response and more vigorous red cell regeneration following parenteral liver therapy than had occurred following the beef muscle or muscle extract, led the authors to assume that there was in these cases deficient absorption from the intestinal tract. In several cases even liver extract fed orally produced an inadequate response whereas the subsequent parenteral injection of liver extract was followed by a much better response. These comparisons were not wholly justified since the doses given parenterally were much larger than those given by mouth; nevertheless some impairment of absorption must be conceded.

By biologic assay in cases of pernicious anemia, it was shown that the gastric juice of 2 of the cases of pellagra actually contained "intrinsic factor." Yet in at least one case it was shown that when normal human gastric juice was fed in addition to beef muscle, a reticulocyte response developed which was greater than had followed feeding of beef muscle alone. This observation indicates that this patient's own stomach had not been producing enough intrinsic factor to utilize completely the extrinsic factor which was provided.

By these experiments, the authors demonstrated that three types of disorder may exist in cases of macrocytic anemia in pellagra and that in some instances all three may operate at the same time. These are (1) a lack of extrinsic factor in the diet, (2) a diminished secretion of intrinsic factor, and (3) faulty absorption from the intestinal tract. In any given case one or more of these factors may operate to a greater or lesser degree.

The authors seek to make a sharp distinction between these cases of macrocytic anemia in pellagra and pernicious anemia. In support of this thesis, they point out that gastric analysis revealed normal gastric acidity in 14 patients, a reduced secretion of hydrochloric acid in 2, and total lack of free hydrochloric acid secretion in 9. In 5 of the last-mentioned cases free hydrochloric acid was found on subsequent examination. In cases of pernicious anemia, with extremely rare exceptions, achlorhydria is found. Again, in pernicious anemia there is often an increase in the quantity of bilirubin in the blood plasma. In the subjects investigated, the icterus index of the plasma was normal or actually reduced. Finally, in pernicious anemia, serum iron values are usually greater than normal. In the cases under study, the serum iron values were either low or within normal limits. Seven of the 25 patients were successfully induced to correct their faulty dietary habits and began eating normal amounts of animal protein. These 7 individuals have remained well and diarrhea and anemia have not reappeared during the two or three years which have since elapsed even though no liver therapy has been given during this time. In pernicious anemia, anemia characteristically returns when liver therapy is neglected although the time interval required varies greatly from case to case.

These studies are of great value in that they reveal the importance of various factors leading to the development of macrocytic anemia. Some, however, will dispute the attempt to make a sharp differentiation from pernicious anemia. Faulty absorption is known to play a role in many cases of pernicious anemia and it is possible that if suitable studies were made, lack of extrinsic factor could be demonstrated to play a part in certain cases as well. The investigators themselves point out that differentiation between their cases and sprue, another condition allied to pernicious anemia, is difficult and may actually not be justifiable.

No explanation is offered for the low serum iron values found in the cases of pellagra. Deficiency of iron in the diet or faulty absorption of iron would seem to be the probable reasons for this finding. Iron deficiency may occur in some cases of pernicious anemia as well as in pellagra (Wintrobe, "Clinical Hematology," *p. 332, Lea & Febiger, Philadelphia (1942)*). It seems probable that if studies were comprehensive enough all grades of deficiency would be found, from "pernicious anemia" where intrinsic factor deficiency plays the major role on the one hand, to the cases of pellagra where dietary lack is perhaps the chief factor, at the other extreme.

It is of interest to note that, unlike the cases of tropical macrocytic anemia described by Wills, hemopoietic responses to highly refined liver extracts occurred in these cases of pellagra. Thus it appears that the nutritional macrocytic anemia found in the United States differs in some fundamental way from the tropical anemia, or the American purified liver extracts contain the hypothetical substance which is lacking in the British anahaemin (Wills and Evans, *Lancet* **II,** *416 (1938)*).

CADMIUM POISONING

Two types of food poisoning have been associated with the ingestion of acid foods or liquids prepared in plated or galvanized metal utensils. The first of these, zinc poisoning, is of rare occurrence. The symptoms are described by Dack ("Food Poisoning," *p. 14, Univ. of Chicago Press, Chicago (1943)*) as "pain in the mouth, throat, and abdomen followed by diarrhea." The outbreaks have most frequently been associated with the cooking of apples in galvanized iron containers.

The second of these metal poisons is that resulting from ingestion of cadmium. The increasing frequency with which this type of intoxication is met is indicated by the data of Fairhall (*Physiol. Rev.* **25,** *182 (1945)*) to the effect that prior to 1941 a total of but 20 cases had been reported in the literature; since January 1941, however, 268 cases have been described. To this latter number should be added 62 cases occurring among Royal New Zealand Air Force personnel as reported by Jenner and Cuningham (*New Zealand Med. J.* **43,** *282 (1944)*).

Practically all the cases of poisoning which have been described may be attributed to the ready solution of metallic cadmium in dilute acids. The airmen mentioned above fell ill immediately after drinking a fruit juice beverage made in a metal urn. Three of the five outbreaks reported by Frant and Kleeman (*J. Am. Med. Assn.* **117,** *86 (1941)*) could be traced to contact of an acid fruit beverage with a cadmium plated refrigerator tray, the fourth to a metal pitcher, and the fifth to replated molds used in the manufacture of popsicles (fruit-flavored ices frozen on a stick).

The symptoms of the poisoning are severe vomiting, acute colicky abdominal pain, and diarrhea, with an onset almost immediately (fifteen minutes to one hour) after ingestion of the soluble cadmium compound. In the severe cases Jenner and Cuningham recorded some degree of shock. The symptoms abate in three to eight hours and recovery within twenty-four to forty-eight hours is the rule.

Jenner and Cuningham estimated that their cases received a total dose of 56 mg. of cadmium. Those cases reported by Frant and Kleeman resulted from ingestion of food contaminated with from 0.013 to 0.53 mg. per gram.

The latter investigators recommend that "no utensils to be used in the preparation or dispensing of food or drink should be permitted to be plated with cadmium." They state: "It is especially important today that the plating industry, searching for substi-

tutes for tin and other valuable war materials, be cautioned that cadmium is not a suitable substitute in the plating of utensils for food."

THE COMPOSITION OF BONE MARROW DURING HEMATOPOIESIS

It was established by Schultze (*J. Biol. Chem.* **138,** *219* (*1941*)) that when the anemia of severe copper deficiency is induced in the rat, the cytochrome oxidase activity of the bone marrow is much reduced, and that the feeding of copper to such rats increased the cytochrome oxidase activity of the marrow to normal levels within twenty-four hours. This observation was extended by Schultze and Simmons (*Ibid.* **142,** *97* (*1942*)) who used radioactive copper and demonstrated that significant amounts of the copper entered the bone marrow within this interval of time. They concluded that copper may exert an essential function within the bone marrow.

In a new study McCoy and Schultze (*J. Biol. Chem.* **156,** *479* (*1944*)) have now presented further chemical studies of the bone marrow in copper deficiency, and during hematopoiesis following the restoration of copper to the diet. The rats were rendered copper (or iron) deficient on suitably prepared diets consisting of hydrogen sulfide-treated raw milk with additions of metallic salts and of thiamine chloride. At various stages of the experiments rats were killed, and long bones (humeri, femurs, and tibias) were dissected out and split lengthwise with a scalpel so that the marrow could be lifted out and suspended in water. Aliquots of the suspension were used in the analyses.

The analyses included the determination of solids, lipids, total nitrogen, ammonia nitrogen, amide nitrogen, urea nitrogen, amino nitrogen, and nonprotein nitrogen, and of hemoglobin in the bone marrow, as well as hemoglobin of the blood. The rats were divided into four groups. The first group contained 9 normal controls, the second, 6 iron-deficient, the third, 11 copper-deficient, and the fourth, 8 rats which had been copper-deficient and then treated with copper for five days. The rats were 10 to 11 weeks of age when killed and examined. The mean hemoglobin levels in the four groups were 13.4, 4.6, 2.9, and 7.65 g. respectively per 100 ml. of blood.

The most striking finding in the composition of the bone marrow was that in the normal control rats the quantity of dry solids in the marrow was considerably higher than in the other three groups, and this was due nearly entirely to the lipid fraction. The other three groups had less than one quarter of the amount of lipid found in the marrow of the normal rats. This large difference makes it necessary, in the opinion of the authors, to express the other determinations on a fat-free solids basis. The nitrogen of the marrow of the normal rats was then found to be lower than in the other three groups, which were at approximately the same level. The same was also true of the solids which were soluble, as well as those insoluble, in water. The marrow of the copper-deficient rats contained less insoluble solids than the marrow of iron-deficient rats or copper-treated rats, and less than those of normal rats.

The amount of hemoglobin found in the marrows was small, but was more than twice as great in the normal or copper-treated rats as in the iron-deficient or copper-deficient rats. On the basis of stated assumptions which are acceptable until measurements are made, the authors calculate that the hematopoiesis occurring during treatment of the previously copper-deficient rats necessitates a complete turnover in the soluble nitrogen of the marrow every sixteen hours.

While this paper is an account of studies incompleted because of changes due to the war, it gives a valuable account of the

several micromethods which the authors devised for the analysis of the bone marrow, and a summary of their findings to the present. Continuation of these studies will, the authors hope, provide evidence about the precursors and types of reactions which are concerned in hemoglobin and red blood cell formation.

CHOLINE IN THE DIETARY THERAPY OF CIRRHOSIS OF THE LIVER

The role of choline in animal and human nutrition has been discussed in this journal (*Nutrition Reviews* **2,** *314* (*1944*)). The preliminary report of Russakoff and Blumberg (*Federation Proceedings* **3,** *96* (*1944*)) has now been followed by a detailed report on the use of choline as an adjuvant to the dietary treatment of cirrhosis in man (*Ann. Int. Med.* **21,** *848* (*1944*)).

Results in 10 patients who were followed for periods up to two years give convincing evidence but not final proof that choline is of definite benefit in the treatment of Laënnec's cirrhosis. It is extremely difficult to make a critical evaluation of any specific therapeutic agent in a disease which has an unknown cause, runs a chronic course, and is subject to "spontaneous" remissions and exacerbations. As knowledge increases, fluctuations in severity and aberrations in the clinical course of a disease may be explained. Relapses and remissions in pernicious anemia, once regarded as "spontaneous," are intelligible now that the pathogenesis is known. Until the specific cause or causes of cirrhosis are established it is not possible to control all factors which might influence the clinical course of the disease while the therapeutic virtue of a single compound or a combination of several are tested. It is necessary to follow a large number of cases for a long time before final proof is obtained.

In cirrhosis certain changes may not be reversed—the fibrous tissue scarring cannot be removed, even by therapy which might prevent cirrhosis or cure it in its earlier stages. It is impossible to detect the stage of fibrosis clinically by signs or by chemical tests of liver function; therefore failures in treatment might result from an irreversible state of fibrosis rather than from insufficiency or nonspecificity of the compound on trial.

For this reason the judgment of the investigator is as important as the statistical validation of results, and a few carefully studied cases may be of value in appraisal of new therapeutic agents. Definite clinical improvement occurred in 7 of the 10 patients reported by Russakoff and Blumberg when choline was added to the high calory, high protein, high carbohydrate, low fat, high vitamin diet. The dosage varied from small quantities up to 6 g. daily. In 3 cases improvement had not occurred on diet alone for three weeks but was definite within the first ten days of choline therapy. Enlarged livers shrank in size, ascites decreased or disappeared, and the general sense of well-being returned. Serum proteins returned to normal from hypoproteinemic levels, liver function as indicated by chemical tests became normal, and the hemogram improved.

While it will be necessary to accumulate a large number of cases before accepting the use of choline as a specific agent in treating human cirrhosis of the liver, the report under consideration suggests that choline be given an extensive and prolonged trial under carefully standardized conditions.

NOTES

Letters to the Editor

Dear Sir:

May I reply to a letter from Dr. Clarence P. Berg in your January issue which comments unfavorably on the work of Albanese and Irby reviewed in your article entitled "Adequacy of Essential Amino Acids" (*Nutrition Reviews* **2,** *263 (1944)*).

Dr. Berg's first point is that the paper of Albanese and Irby should have been ignored because six other reports were at variance with it. Had the conditions in all seven studies been identical this criticism would have been more valid. And, had the other experiments referred to shown no evidence of the trend reported by Albanese and Irby, the criticism would likewise have been more valid. The facts of the case are, however, that the experimental conditions were different, and that somewhat similar trends were observed by several other investigators. It is not difficult to understand how in the presence of a possibly less favorable basal diet, a given set of superimposed conditions might lead to "growth failure" in the hands of Albanese and Irby and merely to "inferior growth" in the hands of other investigators. We may point out that such inferior growth on an essential amino acid mixture was observed by our critic himself (Bauer and Berg, *J. Nutrition* **26,** *51 (1943)*).

The second point raised by Dr. Berg refers to the adequacy of the essential amino acid mixture of Albanese and Irby, particularly with respect to isoleucine, since a mixture of *l*-leucine and *l*-isoleucine of unstated proportions was used. Dr. Berg's query on this point is a pertinent one. At the time our experiments were carried out, a chemical technic showed that this mixture contained between 75 and 80 per cent of the natural *l* (+) isoleucine, a quantity that should have been thoroughly adequate. The results of that chemical analysis, which due to the nature of the procedure, might well have been questioned, have since been confirmed by a bioassay test in the rat (Albanese, *J. Biol. Chem.* **157,** *613 (1945)*).

Dr. Berg's third point concerns the cause of the nutrition failure on Albanese and Irby's essential amino acid diet. In addition to the suggestion which they offered in part and very tentatively—the presence of unnatural isomers not readily utilized—Dr. Berg has other possibilities to suggest, namely: (1) that an excess of some utilizable amino acid may have exercised a deleterious effect and (2) that these animals were not receiving sufficient calcium and phosphorus. It is true that in our mixture some natural amino acids were present in quantities that may be regarded by some as more than optimal, but we may add that we are not aware of anyone who has demonstrated toxic effects at the levels we employed, and we therefore did not regard this possibility as plausible enough to deserve mention. Dr. Berg is correct in pointing out that the Cox-Imboden mineral mixture was originally designed as a supplement for diets in which calcium and phosphorus were otherwise provided, and should be used with a calcium and phosphorus supplement if the mineral intake is to be adequate. In all our experiments such a supplement was used, calcium acid phosphate being added to the salt mixture in the proportion of 25.0 g. per cent. In our other publications we have given the complete composition of the mineral mixture or have stated that the Cox-Imboden mixture was supplemented as above described. However, through an oversight on our part this particular paper failed to mention the calcium acid phosphate supplement. The fact that the supplement was given, was however personally communicated to several interested investigators. In our opinion neither of the explanations offered by Dr. Berg is as satisfactory as that which we originally suggested, and which has since

found some experimental support in published and unpublished work.

The fourth point raised by Dr. Berg implies that the reviewer should have disregarded our work because of the "unorthodox" finding that nitrogen balance did not run parallel to loss of weight. It is obvious, however, that weight is influenced by factors other than nitrogen containing tissue (e.g. by fat and water) and it is also known that nitrogen balance, although a valuable criterion for studying the adequacy of the protein moiety of a diet may under certain circumstances give erroneous information if used as the sole criterion. We note with interest that the phenomenon of weight gain on an inadequate amino acid diet was reported by Bauer and Berg (*J. Nutrition* **26,** *51 (1943)*) who expressed no concern over this "unorthodox" finding.

We are glad that Dr. Berg's comments were published, for by and large our American scientific periodicals are singularly devoid of those little personal discussions that lend color to many a European journal.

Sincerely yours,

ANTHONY A. ALBANESE,
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Johns Hopkins University,
Baltimore, Maryland

Dental Caries and Diet

Since the role of diet in the causation of dental caries is not clearly established, observations bearing on this question are worth noting. Boyd (*Am. J. Dis. Child.* **67,** *278 (1944)*) reports observations on the relative value of diets containing high or low amounts of fat. These observations were made possible by a change in the diet from an original high fat level to one which offered only half as much fat and twice the amount of carbohydrate. The study was made on 59 girls and 52 boys and covers a period of at least eighteen months for each. In the children receiving the "high fat" diet the quantities of fat and carbohydrate were 210 and 90 g. respectively. In those receiving the lower fat diet, the quantities of fat and carbohydrate were 110 and 150 g. respectively. The high fat diets were also higher in caloric allowance and led to some overweight. The lower fat diets provided larger amounts of fruits and tended to provide more ascorbic acid than did the high fat diets. The excess base was greater in the lower fat diets.

The study showed no constant or significant difference in the average rate of progression of caries in the two groups. If diet is a factor in the development of caries, Boyd's data would suggest that the protection against caries depends on factors other than or in addition to those indicated above. His own conclusion is that protection against caries depends on the high nutritive value (in a composite sense) of the contrasted diets.

Nutrition in Great Britain

A recent issue of the British Medical Bulletin (*Vol.* **2,** *nos. 10–11 (1944)*) is devoted entirely to various aspects of nutrition in Great Britain. There are special contributions prepared by Sir Edward Mellanby, Dr. B. S. Platt, and other leaders in nutrition, as well as many brief reviews of selected papers which cover a wide field in experimental nutrition.

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The Editorial Staff has been carefully selected so that the publication will merit in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Assistant Editors.